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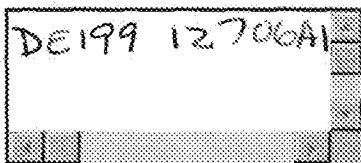


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9/7/2000

The invention relates to DNA sequences of staphylococci, which code for peptides/proteins, which stand with the incorporation of D-alanine in Teichonsäuren in context. Further the invention concerns active ingredients, which can affect the alanine installation in Teichonsäuren, and which are suitable for the treatment bacterial infection illnesses. ←

Nosokomialinfektionen represent a serious problem in the hospital everyday life. Here their transmission simultaneous concerns with the treatment and care made infections, which become frequent caused by banal excitors. By infections with this so-called, Hospital germs become numerous deaths caused. These are based to a large extent on the multiple antibiotic resistances of these seeds. The clinical manifestations of Nosokomialinfektionen are very various. Beside funguses, Salmonellen, enteropathogenen Kolibakterien and clostridiums Grampositive bacteria, as for example, play staphylococci or Streptokokken here a large rôle. A third to the half of all Sepsisfälle caused become by Gram positive bacteria. From the already mentioned staphylococci staphylococcus has aureus the largest pathogenic and thus medical importance. Staphylococci of aureus strains occur with animals and humans and are for example the excitors of abszedierenden inflammations as well as of heart inflammations and the already mentioned sepses.

Although Gram positive bacteria known-measured no endotoxins, form like for the example LPS, they are nevertheless in the layer to cause a septic shock. Due to the often present multiple Antibiotikaresistenzen of these seeds an effective treatment of such infections is not frequent possible.

Lipoteichonsäuren and Zellwandteichonsäuren, in the following as Teichonsäuren designated, are beside the Peptidoglykan major constituents of the cell wall of Gram positive bacteria with low office content (staphylococci, Streptokokken, Bazillen, etc.). The Teichonsäuren can constitute 40-60% of the cell wall of Gram positive bacteria. It concerns here chain molecules with polymere Phosphodiester structure. The Phosphodiester groups are linked with one another by glycerol or Ribit. At an end of the polymere molecule is a Oligosaccharid connecting unit, which carries a Diacylglycerin remainder in the case of the Lipoteichonsäuren again. The Teichonsäuren is substituted with D-alanine and various sugars. Lipoteichonsäuren are anchored over its Glycolipid structure (oligosaccharide + Diacylglycerin) in the cell membrane of the bacteria. The Zellwandteichonsäuren is covalent linked with the Peptidoglykan. The clarity of the structure of the Teichonsäuren becomes on Fig. 1 referred.

The functions of the Teichonsäuren are fully understood so far not yet. It is to be assumed however they are vital for the bacteria. It became an influence of Teichonsäuren on the activity of autolytischen enzymes and on the cell morphology described, which refers to a rôle with the cell division. Besides a control becomes the  $mg < 2+ >$  - concentration in the cell wall by Teichonsäuren discussed. Further is known that Teichonsäuren can possess a Endotoxin similar activity and play a rôle with staphylococcus infections (Kengatharan, K.M., S.De Kimpe, C. Robson, S.J. Foster, and C. Thiemermann. 1998. Mechanism OF Gram positive shock: identification OF peptidoglycan and lipoteichoic acid moieties essential into the induction OF nitric oxides synthase, shock, and multiple organ failure. J. Exp.Med. 188: 305-315).

Due to the reference that Teichonsäuren played an essential rôle for the bacterial cell it was already undertaken, experiments to use the biosynthesis of the Teichonsäuren as possible target point for the search for new active ingredients against staphylococci. The efforts in this direction do not have so far yet to a success guided (W. Fischer, spectrum, special volume 1997, 47-50).

After the prior knowledge conditions the enzymes, which are responsible for the D-alanine-substitution of the Teichonsäuren, are not essential for the bacterial cell (wake, J., Perego, M. and Fischer, W. (1996) Microbial Drug Resistance, volume. 2 on "Mechanism, epidemiology and disease" 123-129). These enzymes did not become therefore so far considered as interesting target points for the active substance development against Gram positive bacteria.

Since up to the present time the need exists, new effective active ingredients for the treatment of diseases, which stand with bacterial infections in context, play Gram positive bacteria a rôle in particular with infections,

The active ingredient according to invention preferably sets with peptides/proteins, which at the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria involved is. The use of such an active ingredient leads accordingly to the fact that the incorporation of D-alanine becomes affected into the Teichonsäuren of the bacteria, whereby a reduction of the D-Alanin Gehaltes of the Teichonsäuren is preferred.

The use of an active ingredient according to invention leads to the fact that the microorganisms, which are to be fought develop a sensitivity or a higher sensitivity opposite antimicrobial substances. With the microorganisms which can be fought it concerns in particular Gram positive bacteria, as for example staphylococci, Streptokokken, Bazillen, etc.

The antimicrobial substances, against which the bacteria after treatment with the active ingredients according to invention one if necessary, increased sensitivity show, can different origin be. Thereby the host of defense peptides can act out of leukocyte around cationic antimicrobial peptides out of human or animal cells, as for the example (Defensine, Protegrine o. A.). Further it can concern also bacterial substances such as Antibiotika. Members of it are for example Gallidermin or nisin.

After the treatment with the active ingredients according to invention the Gram positive bacteria can be eliminated by the antimicrobial substances. Without this corresponding treatment the bacteria are resistant opposite these substances. The antimicrobial substances can be without an application in the body present, as for the example the host of defense peptides from leukocytes from the outside. Also the bacterial Antibiotika can become in the body with presence corresponding bacteria formed. On the other hand it is also possible to supply such antimicrobial peptides one to patients which can be treated.

An active ingredient according to invention can be characterised alternative or additional by the fact that it the bio film formation of microorganisms reduced or prevented. For example staphylococci are aureus and also different Staphylokokkus kinds in the layer, itself on implanted plastic materials, like catheters, heart valves, cardiac pacemakers etc. as a so-called. To settle biofilm, which can lead to consequence-fraught infections up to the life-threatening sepsis. This very unwanted bio film formation of microorganisms, in particular of Staphylokokkus kinds, can be prevented by the invention, in particular by the use of the active ingredient according to invention reduced or even complete.

With the active ingredient according to invention it preferably concerns a peptide or a protein. Also cloths can become out for example natural substance libraries or from combinative libraries used.

The described above active ingredients for the treatment of diseases used, which stand with bacterial infections in context, become according to invention. The so-called form a center of gravity of these infections.

Nosokomialinfektionen, which often arise for example in hospitals. Further an use of these active ingredients comes with the implantation of plastic materials into question, since like the described above bio film formation on such materials by the active ingredients according to invention reduced becomes.

As well as the invention enclosure further pharmaceutical compositions, the corresponding active ingredients contain the use of an active ingredient with at least one of the listed above features to the preparation of a pharmaceutical composition. These pharmaceutical compositions are to be used in particular for the treatment of diseases, which with bacterial infections in context.

Additional one is covered by the invention a method to the treatment of bacterial infections and to the treatment of unwanted bio film formation on implanted materials, whereby the active ingredients according to invention become administered.

Various invention processes can become the search of an active ingredient used, which at least one of the described above features exhibits. Here the sensitivity/sensitivity of the microorganisms which can be fought can be consulted opposite antimicrobial substances as measure for the effect of a potential active ingredient. With corresponding methods the microorganism, for example Gram positive bacteria, which can be fought, becomes with a substance treated, which works antimicrobial. Normally the microorganism would be against such a substance resistant. But by an incubation with a potential active ingredient the sensitivity of the microorganism becomes increased opposite the antimicrobial substance, provided that the active ingredient is effective. This increased sensitivity can become by lowered growth and/or survival rates of the microorganisms which can be fought detected.

An other method for the search of an effective active ingredient against in particular Gram positive bacteria uses the reduced bio film formation at surfaces, in particular at plastic or glass surfaces, as measure for the effect of the potential active ingredient. For this the microorganisms with a potential active ingredient treated and the subsequent bio film formation in form of a determination of schleimbildenden substances, for example the "polysaccharid intercellular adhesin", of Schleimschubstanz general or become analyzed by determining the number of adh rierenden microorganisms, which can be fought. The proof of the bacteria, the Schleimschubstanz or the schleimbildenden substances can take place with dyes, groups of fluorescences, radioactive isotopes or specific antisera.

Favourable also the determination of the binding ability of Teichons uren at surfaces is with addition of, potential active ingredients which can be tested. The proof can take place via mark of the Teichons uren with radioactive isotopes, dyes or groups of fluorescences or become with specific antisera performed.

An other possibility for the search of an active ingredient exists in the analysis of the inflammatory effect of the microorganisms which can be fought. For these studies also the Teichons uren can be consulted, which is altered by the treatment with a potential active ingredient in its alanine content. In order to be able, become to measure the inflammatory effect of the microorganisms and/or the Teichons uren the microorganisms and/or Teichons uren as well as human and/or animal cells and at least a potential active ingredient inkubiert. With the human and/or animal cells it preferably concerns cells, in particular immune cells, which react to a corresponding

with which, to develop, places themselves the invention the object to compile new strategies to the discovery of effective active ingredients and to place on this basis the corresponding active ingredients to the order. The new strategies are based on the results, which became by the provision in the claims 1 to 7 stressed DNA sequences the recovered. The gene products derived of these DNA sequences and/or. Amino acid sequences are stressed in the claims 8 and 10. A vector, which contains corresponding DNA sequences, is in the claims 11 to 14 described. The claims 15 to 17 concern organisms, which are to be won by molecular-biological works with the stressed DNA sequences. Claim 18 concerns Teichonsäuren, which in particular from these organisms formed to become to be able. Claims 19 to 26 describe active ingredients according to invention. The claims 27 and 28 refer itself on the use of these active ingredients and/or. on pharmaceutical compositions to the treatment of bacterial infectious diseases and their use. The methods to the discovery of the active ingredients according to invention are in the claims 29 to 32 shown. The wording of all claims becomes hereby made by reference the content of the description.

The invention covers the surprising result that with Gram positive bacteria the alanine installation stands in Teichonsäuren with the sensitivity of these bacteria opposite antimicrobial substances in the context. The substitution of the Teichonsäuren with alanine conditional the obvious resistance of the bacteria against such substances. These meant that by an inhibition of the alanine installation the bacteria become more sensitive opposite antimicrobial substances, and are in this way open to attack. This result opens a complete new aspect for a Wirkstofffindung, as the alanine installation in Teichonsäuren becomes a chosen as point of attack for the active ingredients which can be developed.

Contrary to the highly specific resistance systems of the Lantibiotika the D-Alaninester with a general resistance mechanism seems to stand against cationic peptides in the context. D-Alaninester, which play themselves with many other pathogenic bacteria, as Streptokokken, Enterokokken and Listeria find, thus an important role with the resistance of these pathogens opposite the components of the natural immunity.

The genes, which code C2a and staphylococcus for peptides/proteins, which are involved at the incorporation of D-alanine in Teichonsäuren, became in staphylococci xylosus aureus Sa113 identified and sequenziert (Fig. 2 and Fig. 3). The DNA sequences possess the same arrangement in both organisms and are present in a opera-on-similar structure with subsequent Transkriptionsterminator. The structure of this so-called. DltABCD operon goes out of the Fig. 4, upper part, out. By the corresponding sequences four peptides/proteins encoded, DltA, become i.e. DltB, DltC, DltD. An other open reading frame (orf 1) to 5' - end of the sequence exhibits similarities to Hydroxysäure dehydrogenases. DltA is a D-alanine-D Alanyl Carrierproteinligase. DltB is probably a membrane protein, which also at the D-Alanineinbau in Teichonsäuren involved is. DltC is a D-alanine-Carrierprotein and a DltD is probably a Exoprotein, which likewise at the D-Alanineinbau in Teichonsäuren involved is (Fig. 5 and 6).

With the identification and characterization of the dlt genes from staphylococci xylosus and staphylococci aureus also the corresponding homologous genes from other staphylococcus kinds and used kinds can be identified.

Beside the DNA sequences of these dltABCD operons, including their fragments, from staphylococci xylosus and aureus the invention DNA sequences, which hybridize with these sequences, covers staphylococci.

Further the invention DNA sequences dltABCD of the operons covers, those at least a mutation inertial. Examples for such mutations are insertions or deletions. By Transposoninsertionen and homologous recombination using common molecular-biological methods the importance of the DNA sequences according to invention for the incorporation of D-alanine became in Teichonsäuren and the importance this D of alanine installation for the bacteria investigated, as is to be inferred from the examples. The mutation strategies, which became exemplarily performed, go out of Fig. 4 out.

The invention covers these sequences or parts of it beside the DNA sequences vectors for for example molecular-biological works, inertial. In particular hereby vectors are meant, which exhibit a corresponding DNA sequence in altered form, whereby for example is prevented by an insertion or a deletion the expression of a functional peptide/protein. The expression of the peptides/proteins can become also by an intervention into the regulatorischen elements of the sequence, preferred promoter sequences corresponding by an inactivation, affected. By the use of these vectors according to invention the operation of the various, in the dltABCD Operonkodierten proteins/peptides can become investigated and targeted affected.

Further organisms are covered by the invention, whereby it concerns here in particular bacterial microorganisms, as for example Gram positive bacteria, concerning. the peptides/proteins involved at the D-Alanineinbau altered are. Here it can concern for example that one of these peptides/proteins in particularly potent mass becomes formed. It is also possible to decrease the formation of a peptide/a protein targeted or prevent complete. The altered express ion rates of the corresponding peptides/proteins can in particular become thereby achieved that the relevant genes inactivated to become, altered in their number of copies to become or that the Transkriptions or translation signals altered becomes.

The invention refers also to the Teichonsäuren and/or. their forerunner molecules, which become formed of these altered organisms, whereby this Teichonsäuren differs according to invention by its D-Alaniningehalt from natural occurring Teichonsäuren. Such corresponding altered Teichonsäuren can become also through in vitro methods prepared.

An active ingredient according to invention is characterised in that it an effect, for example in the human body, standing with Teichonsäuren in the context, affected, whereby a reduction of an undesirable effect is preferred. Teichonsäuren as ingredients of the cell wall/cell membrane of Gram positive bacteria can exercise a inflammation-promoting, thus inflammatory, effect on human or animal cells. This for the body usually deleterious effect is switched off by the active ingredient according to invention reduced or.

stimulation more measurable. The measurement of the inflammatory effect of the microorganisms and/or Teichonsäuren which can be fought can become by the determination of a cytokine, a surface marker, a signal transduction component or by the determination of the Degranulations and/or the Phagocytoseeffizienz made. The selection of the signal which can be determined depends on the chosen cell type. The smaller the measured signal after treatment with a potential active ingredient is, the more effective is the active ingredient.

Finally a method can become the active substance search used, which uses the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria as measure for the effect of the potential active ingredient. The measurement of the alanine installation can take place direct at the Teichonsäuren after treatment of the microorganisms with a potential active ingredient, or it can become the connection of D-alanine to enzymes of the alanine installation and/or the conversion of D-alanine by enzymes of the D-Alanineinbaus certain. The proof of the D-alanine or the enzymes can take place with radioactive isotopes, with coloring material or groups of fluorescences, or with antisera, which recognize specific alanine-substituted or non-substituted Teichonsäuren. Also antisera used cannot become, which recognize specific enzymes, the alanine bound or bound to have.

In order to develop a potential active ingredient, which can become for example tested in one of the described above methods, it is meaningful to proceed from one of the peptides/proteins which are involved at the D-Alanineinbau in Teichonsäuren. For example using computer Modelling methods then, first theoretical, an active ingredient developed, becomes which competes with the natural peptides/proteins around a reciprocal effect partner, for the example a substrate, however not the biological function of the natural peptides/proteins met. Such an active ingredient can decrease the biological activity of the natural peptides/proteins involved at the D-Alanineinbau in effective manner or restrain complete.

Another possibility of the active substance design is to select a peptide/a protein of the D-Alanineinbaus than point of attack for the active ingredient which can be developed. For example by computer based models a substance can become developed, which interacts with the natural peptide/protein and restrains this. The potential active ingredients developed on basis of these theoretical Design considerations then tested can become in one of the invention processes specified above on their efficacy.

Further the active ingredients which can be tested can originate as test substances from natural substance libraries, Peptidbibliotheken, phage display libraries or similar.

The described features and other features of the invention result from the figs and the subsequent description of examples in compound with the Unteransprüchen. Here the single features can be in each case for itself or to several in combination realized with one another.

The figs show:

Fig. 1 schematic structure of Lipo and Wandteichonsäuren. With the Wandteichonsäuren of *S. aureus* first three Glycerolphosphateinheiten, followed of approx. follow after the connecting unit. 40 Ribitolphosphateinheiten. With the Wandteichonsäuren of *S. xylosus* Glycerol and Ribitolphosphateinheiten in the same proportion are present. MurNAc: N- Acetylmuraminsäure; GlcNAc: N-acetylglucosamine.

Fig. 2 DNA sequence of the *dltABCD* operon of staphylococci *xylosus* C2a.

Fig. 3 DNA sequence of the *dltABCD* operon of staphylococci *aureus* Sa113.

Fig. 4 structure and inactivation that of *dlt* operon of *S. aureus* and *S. xylosus*. The positions of Transposoninsertionen into the *dlt* operon of *S. xylosus* (XG4-XG24) shown are in the upper part. The *dltA* gene of *S. aureus* *dlt1* became against a Spectinomycin Resistenzkassette (*spc*) exchanged by homologous recombination using the Plasmides represented down pBT. T: Transkriptionsterminator.

Fig. 5 AS-sequences and functions of the peptides/proteins encoded of *dltABCD* the operon of staphylococci *xylosus*. The position indications refer itself on the DNA sequence from Fig. 2.

Fig. 6 AS-sequences and functions of the peptides/proteins encoded of *dltABCD* the operon of staphylococci *aureus*. The position indications refer itself on the DNA sequence from Fig. 3.

Fig. 7 tabular representation of the D-Alaninergehalts of the Teichonsäuren of *S. aureus* and *S. xylosus* (wild type and *dlt* mutants).

Fig. 8 tabular representation of the activity of antimicrobial peptides against the trunks of type of game *S. aureus* Sa113 and *S. xylosus* C2a as well as the *dlt* mutants *S. aureus* AG1 and *S. xylosus* XG13.

Fig. 9 graph of the connection of anionic and cationic proteins by *S. aureus* and *S. Xylosus* strains. Wild type (A, black beams), *dltA* mutants (b, white beams) and wild type with pRBdlt1 (C, grey beam) were inkubiert with neutralem pH with various proteins and the amount at unbound protein in the culture projection became certain.

Examples

#### 1. Methods

*S. aureus* Sa113 (Iordanescu, S. and Surdeanu, M. (1976) J. Gene. Microbiol. 96, 277-281), *S. xylosus* C2a (Brückner, R. (1997) FEMS Microbiol. Lett. 151, 1-8) and *E. coli* DHSa (Ausubel, F.M., Brent, R., Kingston, R.E., Woorlands, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1990) Current protocols in molecular biology, John Wiley and Sons, Inc., New York, N.Y.) became cultured in CBM Mediums (1% Trypton, 0.5% yeast extract, 0.5% NaCl, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.1% glucose), as long as nothing else is indicated.

The plasmid pTV1ts, which for the Transposon mutagenesis used becomes, contains a temperature-sensitive replicon, a chloramphenicol resistance gene and the transposon Tn917, which a Erythromycin resistance mediated (Youngman, P., Poth, H., Green, B., York, K., Olmedo, G. and Smith, K. (1989) in regularization OF procaryotic development (Smith, I., Slepacky, R.A. and Setlow, P., eds), pages 65-87, American Society for

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Microbiology, Washington, DC). In order to manufacture a collection of mutants, *S. xyloso* (pTV1ts) over night with 30 DEG C in CBM Mediums cultured, which 5  $\mu$ g Erythromycin/ml and 20  $\mu$ g Chloramphenicol/ml contained. The culture became subsequent 100-fach with CBM Mediums diluted, which 2.5  $\mu$ g Erythromycin/ml contained, and for 14 hours with 42 DEG C inkubiert, in order to selektionieren Transposon Insertionsmutanten. This culture became an other time in the similar manner diluted and for other 14 hours with 42 DEG C cultured. Aliquots of the bacteria suspension were deleted on CBM agar plates, which 2.5  $\mu$ g contained Erythromycin/ml, and inkubiert with 37 DEG C. Mutanten clones (4000) became selected on CBM agar plates, which 3 contained  $\mu$ g Gallidermin/ml, transferred and regarding deteriorated growth on Gallidermin.

DNA became by cycle sequencing (Ausubel, F.M., Brent, R., Kingston, R.E., moorlands, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1990) Current protocols in molecular biology, John Wiley and Sons, Inc., New York, N.Y.) in a DNA Sequenzierungsgerät 4000 L (LI-COR Inc., Lincoln, Neb., the USA) sequenziert, whereby that became Thermosequenzasefluoreszenz labeled cycle sequencing kit (Amersham, Little Chalfont, UK) used.

Around the *dltA* gene of *S. aureus* Sa113 by a Spectinomycin resistance gene to replace, became DNA fragments of 1.5 KB each, which flank *dltA*, by PCR amplified, over the *Sma*I interface of pUC18 in accordance with standard methods cloned (Ausubel, F.M., Brent, R., Kingston, R.E., moorlands, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1990) Current protocols in molecular biology, John Wiley and Sons, Inc., New York, N.Y.) and subsequent sequenziert. By cuts with *Sph*I/*Sac*I and/or. *Bam*HI/*Eco*RI became up and downward-located fragments from the resultant plasmid isolated and into the temperature-sensitive plasmid the pBT2 (Brückner, R. (1997) FEMS Microbiol. Lett. 151, 1-8) together with that 1277 BP *Sac*I *Bam*HI fragment inserted, which for a Spectinomycin adenyl transferase gene (*spc*) of Tn554 (Murphy, E., Huwyler, L. and de Freire Bastos, M.d.C. (1985) EMBO J. 4, 3357-3365) encoded, as in Fig. 4, bottom, shown is.

The resultant plasmid pBT DELTA *dlt1* became in *S. aureus* Sa113 by electroporation introduced (Augustin, J. and Götz, F. (1990) FEMS Microbiol. Lett. 66, 203-208). By incubation with 42 DEG C and subsequent search for Spectinomycin resistant clones without Plasmid encoded chloramphenicol resistance, the mutant AG1 identified, which contains the *spc* gene instead of the *dltA* gene, became. The correct integration of *spc* became by sequencing the DNA verified.

The plasmid pRBdlt1 became by ligation 4655 bp-PCR of a fragment, which the *dltABCD* operon of *S. xyloso* C2a covered, as well as 393 BP upward from the *dltA* start codon with the putative activator region and 185 BP downward the *dltD* stop codon with the terminator structure into the *Sma*I interface of pUC18 constructed. After the sequence analysis the fragment became isolated by cuts with *Bam*HI *Eco*RI from the resultant plasmid, into the multiple cloning site of the vector pRB473 (Brückner, R. (1992) Genes 122, 187-192) cloned and in *S. xyloso* C2a and *S. aureus* Sa113 by electroporation introduced (Augustin, J. and Götz, F., (1990) FEMS Microb. Lett. 66, 203-208).

For complete act ion experiments mutants with the plasmid became pRBdlt1 transformed. For this protoplasts of mutants with protoplasts of type of game, which contained this plasmid, fused and subsequent became on the Spectinomycin resistance (*S. aureus*) or Erythromycin resistance (*S. xyloso*) and the Plasmid encoded Cloramphenicol resistance selektioniert. The Protoplasten fusion became as in Götz, F., Ahrne, S. and Lindberg, M. (1981) J. Bacteriol. 145, 74-81 described, performed. The resultant clones became checked by analysis of restriction fragments and DNA sequencing.

For the isolation of the Teichonsäuren the bacteria over night in 500 became ml CBM Mediums, which 0.25% (*S.*

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1. DNA sequence of staphylococci xylosus in accordance with Fig. 2 or fragments of it.
2. DNA sequence of staphylococci aureus in accordance with Fig. 3 or fragments of it.
3. DNA sequence, characterised in that it bottom stringent conditions with the DNA sequence in accordance with Fig. 2 or with the DNA sequence in accordance with Fig. 3 or fragments of it hybridized.
4. DNA sequence, characterised in that it for at least a peptide/protein with in Fig. 5 or Fig. 6 indicated amino acid sequences encoded.
5. DNA sequence after one of the preceding claims, characterised in that it at least a mutation, in particular an insertion or a deletion, exhibits, whereby a mutation is preferably in present for peptides/proteins coding section of the sequence.
6. DNA sequence according to claim 5, characterised in that at least one of the peptides/proteins in its biological function, encoded of the DNA sequence, disturbed, in particular inactivated, is.
7. DNA sequence after one of the preceding claims, characterised in that the peptide/the protein at the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria involved, encoded of the DNA sequence, is.
8. Gene product derived from at least a DNA sequence to one of the preceding claims.
9. Peptide/protein with one in the Fig. 5 or 6 indicated amino acid.
10. Peptide/protein according to claim 9, characterised in that it at the incorporation of D-alanine in Teichonsäuren of Grampositiven bacteria involved is.
11. Vector, characterised in that it at least a part of a DNA sequence after one of the claims 1 to 7, in particular after one of the claims 5 and 6, exhibits.
12. Vector according to claim 11, characterised in that it at least for a peptide/a protein coding section of a DNA sequence exhibits.
13. Vector according to claim 12, characterised in that the coding section at least a mutation exhibits.
14. Vector after claims 12 or claim 13, characterised in that the peptide/protein at the incorporation of D alanine in Teichonsäuren of Gram positive bacteria involved is.
15. Organism, in particular bacterial microorganism, characterised in that the organism an altered expression of at least one of the genes exhibits, which code for peptides/proteins, which are at the incorporation of D alanine in Teichonsäuren involved.
16. Organism according to claim 15, characterised in that at least one of the genes over-expressed is.
17. Organism according to claim 15 or claim 16, characterised in that at least one of the genes in its expression reduced, preferred switched off, is.
18. Teichonsäure, which is of an organism in accordance with one of the claims 15 to 17 formed and/or is in its structure opposite natural occurring Teichonsäuren altered.
19. Active ingredient, that direct and/or the indirect effect of Teichonsäuren, in particular their inflammatory effect on cells, in particular on human and/or animal cells, affected, preferably reduced.
20. Active ingredient according to claim 19, characterised in that the active ingredient the incorporation of D-alanine in Teichonsäuren affected, preferably reduced.
21. Active ingredient according to claim 19 or claim 20, characterised in that the active ingredient at least a peptide/a protein, which at the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria involved is, in its activity affected, preferably restrains.
22. Active ingredient, in particular after one of the claims 19 to 21, characterised in that the active ingredient the sensitivity of microorganisms opposite antimicrobial substances affected, preferably increased.

23. Active ingredient according to claim 22, characterised in that the microorganisms bacteria are, in particular Grampositive bacteria.

24. Active ingredient according to claim 22 or claim 23, characterised in that it with the antimicrobial substances around cationic antimicrobial peptides, in particular host of defense peptides and/or bacterial antimicrobial peptides acts.

25. Active ingredient, in particular after one of the claims 19 to 24, characterised in that it the bio film formation of microorganisms, in particular staphylococci, at surfaces, in particular glass, metal or plastic surfaces, affected, preferably reduced.

26. Active ingredient after one the Ansprüche 19 to 25, characterised in that the active ingredient a peptide or a protein is.

27. Pharmaceutical composition, characterised in that it at least an active ingredient with at least one of the features in accordance with at least one of the claims 19 to 26 in an effective amount as well as preferably at least a pharmaceutical carrier contains.

28. Use at least an active ingredient after at least one of the claims 19 to 26 or the pharmaceutical composition according to claim 27 to the treatment of diseases, which stand with bacterial infections in context.

29. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the sensitivity of microorganisms opposite antimicrobial substances as measure for the effect of the potential active ingredient certain becomes, comprising

- a) a treatment of microorganisms with at least an antimicrobial substance,
- b) an incubation with at least a potential active ingredient and
- c) the determination of growth and/or survival rates of the microorganisms.

30. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the bio film formation of microorganisms as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms with at least a potential active ingredient and
- b) the determination of at least a schleimbildenden protein and/or the determination of Schleimsubstanz and/or the determination of adhärierenden microorganisms.

31. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the inflammatory effect of microorganisms and/or Teichonsäuren as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms and/or Teichonsäuren as well as human and/or animal cells with at least a potential active ingredient and
- b) the determination of at least a cytokine and/or at least one surface marker and/or at least one signal transduction component and/or the determination of the Degranulations and/or the Phagozytoseeffizienz.

32. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the incorporation of D-alanine in Teichonsäuren as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms with at least a potential active ingredient and
- b) the determination of the installation of D-alanine and/or the connection of D-alanine to enzymes of the D-Alanineinbaus and/or the conversion of D-alanine by enzymes of the D-Alanineinbaus.

1. DNA sequence of staphylococci xylosus in accordance with Fig. 2 or fragments of it.
2. DNA sequence of staphylococci aureus in accordance with Fig. 3 or fragments of it.
3. DNA sequence, characterised in that it bottom stringent conditions with the DNA sequence in accordance with Fig. 2 or with the DNA sequence in accordance with Fig. 3 or fragments of it hybridized.
4. DNA sequence, characterised in that it for at least a peptide/protein with in Fig. 5 or Fig. 6 indicated amino acid sequences encoded.
5. DNA sequence after one of the preceding claims, characterised in that it at least a mutation, in particular an insertion or a deletion, exhibits, whereby a mutation is preferably in present for peptides/proteins coding section of the sequence.
6. DNA sequence according to claim 5, characterised in that at least one of the peptides/proteins in its biological function, encoded of the DNA sequence, disturbed, in particular inactivated, is.
7. DNA sequence after one of the preceding claims, characterised in that the peptide/the protein at the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria involved, encoded of the DNA sequence, is.
8. Gene product derived from at least a DNA sequence to one of the preceding claims.
9. Peptide/protein with one in the Fig. 5 or 6 indicated amino acid.
10. Peptide/protein according to claim 9, characterised in that it at the incorporation of D-alanine in Teichonsäuren of Grampositiven bacteria involved is.
11. Vector, characterised in that it at least a part of a DNA sequence after one of the claims 1 to 7, in particular after one of the claims 5 and 6, exhibits.
12. Vector according to claim 11, characterised in that it at least for a peptide/a protein coding section of a DNA sequence exhibits.
13. Vector according to claim 12, characterised in that the coding section at least a mutation exhibits.
14. Vector after claims 12 or claim 13, characterised in that the peptide/protein at the incorporation of D alanine in Teichonsäuren of Gram positive bacteria involved is.
15. Organism, in particular bacterial microorganism, characterised in that the organism an altered expression of at least one of the genes exhibits, which code for peptides/proteins, which are at the incorporation of D alanine in Teichonsäuren involved.
16. Organism according to claim 15, characterised in that at least one of the genes over-expressed is.
17. Organism according to claim 15 or claim 16, characterised in that at least one of the genes in its expression reduced, preferred switched off, is.
18. Teichonsäure, which is of an organism in accordance with one of the claims 15 to 17 formed and/or is in its structure opposite natural occurring Teichonsäuren altered.
19. Active ingredient, that direct and/or the indirect effect of Teichonsäuren, in particular their inflammatory effect on cells, in particular on human and/or animal cells, affected, preferably reduced.
20. Active ingredient according to claim 19, characterised in that the active ingredient the incorporation of D-alanine in Teichonsäuren affected, preferably reduced.
21. Active ingredient according to claim 19 or claim 20, characterised in that the active ingredient at least a peptide/a protein, which at the incorporation of D-alanine in Teichonsäuren of Gram positive bacteria involved is, in its activity affected, preferably restrains.
22. Active ingredient, in particular after one of the claims 19 to 21, characterised in that the active ingredient the sensitivity of microorganisms opposite antimicrobial substances affected, preferably increased.
23. Active ingredient according to claim 22, characterised in that the microorganisms bacteria are, in particular Grampositive bacteria.
24. Active ingredient according to claim 22 or claim 23, characterised in that it with the antimicrobial substances around cationic antimicrobial peptides, in particular host of defense peptides and/or bacterial antimicrobial peptides acts.
25. Active ingredient, in particular after one of the claims 19 to 24, characterised in that it the bio film formation of microorganisms, in particular staphylococci, at surfaces, in particular glass, metal or plastic surfaces, affected, preferably reduced.
26. Active ingredient after one the Ansprüche 19 to 25, characterised in that the active ingredient a peptide or a protein is.
27. Pharmaceutical composition, characterised in that it at least an active ingredient with at least one of the features in accordance with at least one of the claims 19 to 26 in an effective amount as well as preferably at least a pharmaceutical carrier contains.
28. Use at least an active ingredient after at least one of the claims 19 to 26 or the pharmaceutical composition according to claim 27 to the treatment of diseases, which stand with bacterial infections in context.
29. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the sensitivity of microorganisms opposite antimicrobial substances as measure for the effect of the potential active ingredient certain becomes, comprising

- a) a treatment of microorganisms with at least an antimicrobial substance,
- b) an incubation with at least a potential active ingredient and
- c) the determination of growth and/or survival rates of the microorganisms.

30. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the bio film formation of microorganisms as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms with at least a potential active ingredient and
- b) the determination of at least a schleimbildenden protein and/or the determination of Schleimsubstanz and/or the determination of adh rierenden microorganisms.

31. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the inflammatory effect of microorganisms and/or Teichons uren as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms and/or Teichons uren as well as human and/or animal cells with at least a potential active ingredient and
- b) the determination of at least a cytokine and/or at least one surface marker and/or at least one signal transduction component and/or the determination of the Degranulations and/or the Phagozytoseeffizienz.

32. Method to the provision of an active ingredient with at least one of the features after at least one of the claims 19 to 26, characterised in that the incorporation of D-alanine in Teichons uren as measure for the effect of the potential active ingredient certain becomes, comprising

- a) an incubation of microorganisms with at least a potential active ingredient and
- b) the determination of the installation of D-alanine and/or the connection of D-alanine to enzymes of the D-Alanineinbaus and/or the conversion of D-alanine by enzymes of the D-Alanineinbaus.

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TITLE: New staphylococcal DNA for dltABCD operons, useful e.g. for identifying antibacterials and agents that reduce bacterial resistance to antimicrobials

INVENTOR: GOETZ F ; PESCHEL A

PATENT-ASSIGNEE: PETRY GENMEDICS GMBH (PETRN)

PRIORITY-DATA: 1999DE-1009636 (March 5, 1999)

Search Selected

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PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
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APPLICATION-DATA:

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INT-CL-CURRENT:

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ABSTRACTED-PUB-NO: DE 19912706 A1

BASIC-ABSTRACT:

NOVELTY - DNA sequences (A) of the dltABCD operons from Staphylococcus xylosus (about 5.4 kilobases; 2) and S. aureus (about 5.9 kilobases; 3), both reproduced, are new.

DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (a) DNA sequences (A') that hybridize to (2), (3) or their fragments under stringent conditions;
- (b) DNA sequences (A'') that encode any of 10 specified peptide sequences, reproduced;
- (c) DNA sequences (B) derived from (A), (A') or (A'') by at least one mutation, particularly an insertion or deletion, especially in the coding region;
- (d) gene products (C) from (A), (A'), (A'') and (B);

- (e) peptides (D) specified in (b);
- (f) vectors containing (A), (A'), (A'') and (B);
- (g) organisms, particularly bacteria, that have altered expression of at least one gene that encodes a protein involved in incorporation of **D-alanine** (Dala) into **teichoic** acid (I);
- (h) (I) expressed in the organisms of (g) and/or having a structure different from that of wild-type (I);
- (i) an active agent (II) that (in)directly modulates or specifically reduces, the activity of (I), especially its inflammatory activity on cells;
- (j) an active agent (II') that modulates or specifically increases, the sensitivity of microbes to antimicrobial agents;
- (k) a pharmaceutical composition containing at least one (II) or (II') and optionally a carrier; and
- (l) a method for identifying (II) or (II').

The proteins expressed by (A) are involved in incorporation of **D-alanine** (Dala) into **teichoic** acid (I) (which has endotoxin-like inflammatory activity) by Gram-positive bacteria. Incorporation of Dala into (I) is correlated with sensitivity of bacteria to antimicrobial agents, i.e. Dala is necessary for resistance. When tested against wild-type *S. aureus* Sal13, the human neutrophilic peptide defensin had minimum inhibitory concentration (MIC) over 100 micrograms/milliliter, but against a mutant in which the *dltA* gene has been deleted it had MIC over 10 micrograms/milliliter. Similar reductions in MIC were determined for other cationic antibacterial peptides.

USE - (A), optionally mutated, are used to study the function of their encoded proteins, involved in incorporation of **D-alanine** (Dala) into **teichoic** acid (I). Agents that reduce the inflammatory activity of (I) or incorporation of Dala into (I) are used:

- (i) to increase the sensitivity of Gram-positive bacteria to antimicrobial agents;
- (ii) to inhibit formation of biofilms (particularly of staphylococci) on glass, metal or plastics surfaces (e.g. catheters or cardiac pacemakers); and
- (iii) as antibacterials.

ABSTRACTED-PUB-NO: DE 19912706 A1  
EQUIVALENT-ABSTRACTS:

## BIOTECHNOLOGY

Preferred Mutations: The mutations result in a protein with disrupted, especially inactivated, function.

Preferred Proteins: The specification includes deduced amino acid sequences, from both staphylococcal species, for:

- (i) *orf1*, similar to hydroxyacid dehydrogenase;

- (ii) DltA, D-alanine-D-alanyl carrier protein ligase;
- (iii) DltB, probable membrane protein involved in incorporation of Dala into (I);
- (iv) DltC, Dala carrier protein; and
- (v) DltD, probable exoprotein involved in Dala incorporation into (I).

## BIOLOGY

Preferred Materials: (II) inhibit incorporation of Dala into (I) in Gram-positive bacteria and (IIa) is especially a cationic antimicrobial peptide, e.g. a human defense peptide and/or bacterial antimicrobial peptide.

Preferred Process: To identify (II) or (IIa):

- (i) a microbe is treated with an antimicrobial agent, then incubated with a test compound and the growth/survival of the microbe measured;
- (ii) the microbe is incubated with a test compound and formation of a slime-forming protein (or compound) and/or microbial adherence is measured;
- (iii) microbes and/or (I) are incubated with human or animal cells and a test compound, then a cytokine, surface marker, or signal transduction compound, and degranulation and phagocytic activity is measured, or
- (iv) microbes are incubated with a test compound, then at least one of incorporation of Dala into (I), binding of Dala to enzymes that incorporate it and/or conversion of Dala by such enzymes is measured.

DERWENT-CLASS: B04 D16

CPI-CODES: B04-B01B; B04-C01; B04-C03; B04-E02F; B04-E03F; B04-E08; B04-F1100E; B04-L08; B04-N03; B11-C08E; B12-K04E; B14-A01; B14-C03; B14-L01; B14-L06; D05-H09; D05-H12A; D05-H12B2; D05-H12E; D05-H14A1; D05-H17A3; D05-H17A6; D05-H17B3; D05-H17B6;

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